In vivo and *in vitro* study of immersion clearing dynamics of the skin

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ABSTRACT

Experimental results on *in vivo* and *in vitro* immersion clearing dynamics of the skin under action of the osmotic agent are presented. The significant decrease of the human skin reflectance *in vivo* under action of *glucose* solution is demonstrated. *In vitro* experiments with dyed immersion agents have shown that the immersion mostly penetrates into skin tissue through the dermal layer. Diffusion of the immersion agent through the stratum corneum barrier was insignificant. It was shown that hypodermic injection of the immersion agent is an effective way for the agent administration. The time-dependent contrast of the blood perfused areas in the cleared skin was estimated. The diffusion coefficient of 40%-*glucose* solution in skin *in vivo* was estimated as $D = (2.56 \pm 0.13) \cdot 10^{-6}\ cm^2/\sec$.

Keywords: optical properties, human skin, *glucose*, refractive index matching, diffusion coefficient.

1. INTRODUCTION

The control of tissue optical properties is important for development of optical tomography, photodynamic therapy and selective photodamage of tissue components. As a scattering medium, tissue shows optical effects that are characteristic for turbid physical systems. Administration of the appropriate chemical agents, like solutions of *glucose*, *glycerol*, *propylene glycol*, *etc.* can effectively change scattering properties of living tissues. Experimental studies on optical clearing of normal and pathological skin and its components (epidermis and dermis) and the management of reflectance and transmittance spectra using *water*, *glycerol*, *glycerol-water* solutions, *glucose*, sunscreen creams, cosmetic lotions, gels and pharmaceutical products were presented in [1-5].

The control of skin optical properties relates to the immersion of refractive indices of scatterers and ground matter. Skin optical properties in general are defined by dermis because of relatively big thickness of dermis (95% of the human skin). The dermis consists mainly of network of collagen fibers, elastic fibers, and an interstitial substance consisting of proteoglycans, salts, and water. Refractive indices of skin components differ from each other and from that of interstitial medium. The variation in refractive index causes a high scattering within the dermis, that destroys the laser light beams used in therapy and surgery and collapses images of skin abnormalities. For example, the main scatterers of the dermis (hydrated collagen fibers) have refractive index n_c=1.46 and that of interstitial medium is n_i=1.36.

Osmotic agents like *glucose* solutions, *dimethyl sulfoxide* solution, *Trasograph*, *Verografin*, *glycerol*, *propylene glycol*, *etc.* have refractive indices close to that of collagen and can be used to alter the scattering properties of tissues [3-12]. Tissue – osmotic liquid interaction accompanies by a change of tissue thickness, sizes, and packing density of scatterers, but the refractive index matching effect prevails over other processes. As the secondary effects, drying of connective tissue fibers [3] and cells swelling or shrinkage are observed [4,5,10-12].

The present study illustrates the effects that 40%-*glucose* solution has on the scattering properties of skin *in vivo*. Diffusion of dye dissolved in osmotic liquids into skin tissue *in vitro* has been studied. The diffusion coefficient of 40%-*glucose* solution in human skin *in vivo* has been estimated.

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2. MATERIALS AND METHODS

The measurements of the reflectance spectra were performed using the commercially available spectrometer LESA-5 (BioSpec, Russia). The scheme of the experimental setup is shown in Fig. 1. *In vivo* reflectance measurements were performed using the fiber optical probe with system of optical fibers, which can be presented as the system of two fibers (radiation source and detector) with equivalent distance between them r_{sd} = 2.5 mm and the probing depth $0.35r_{sd}$ = 0.9 mm [9]. All fibers are enclosed in aluminum jacket (6-mm outer diameter) to provide a fixed distance between the fibers and the sample surface. The reflectance spectra of the samples were measured against $BaSO_4$ plate as a reference. Recording of reflectance spectra of the human skin was provided by placing the fiber optical probe on the surface of the skin. The measurements were performed every 60 sec for 140 min. All experiments were performed at room temperature.

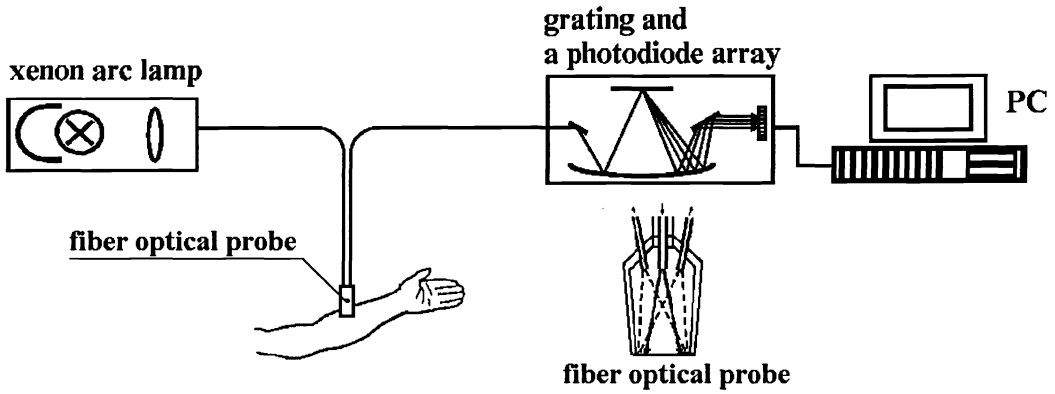

Fig. 1: Experimental setup for measurements of the reflectance spectra *in vivo*.

The volunteer's skin was used for *in vivo* experiments. Hypodermic injection of 40%-*glucose* solution by volume of 0.1 ml was done in the area of forearm. Measurements of the reflectance spectra of the human skin were done in the wavelength range 400 – 800 nm.

The rat skin samples were used for *in vitro* experiments. The samples were put into 40%-*glucose* solution or *glycerol* dyed by *Methylene Blue*. Refractive indices of both *glucose* solution and *glycerol* have been measured as 1.396 and 1.454 at wavelength 589 nm, *pH* of that agents have been measured as 3.5 and 6.5, respectively. Skin specimens were placed into cuvettes with osmotic liquids for 24 hours. They were taken out from cuvettes after different time intervals. Cross-section cuts of samples were done. The first cut was done after three hours of the agent action. Photographs of the cross-section cuts in different time intervals were done by a color imaging system composed of a video-microscope interfaced with a personal computer.

3. RESULTS AND DISCUSSION

3.1. *In vivo* study of the optical clearing of the human skin

Figures 2 and 3 show dynamics of reflectance spectra and time-dependent reflectance at different wavelengths of *in vivo* human skin injected by 40%-*glucose* solution. It can be noted that the effect of tissue clearing was similar to that seen in excised skin specimens [4,5]. From the spectrum of reflectance of the skin it is well seen the main absorption bands of blood (Fig.2).

In Fig. 3, abrupt decrease and followed gradual increase of the reflectance in the first twenty minutes are seen. They are explained by the change of experimental geometry due to appearance of swelling of skin surface after hypodermic injection and then return back of the skin surface to the approximately initial state. Then slow diffusion of *glucose* solution up to the skin surface and corresponding tissue clearing took place. The *glucose*-injected region became more transparent. The area around the injection site that was unaffected by the *glucose* solution remained white and turbid.

Reflectance of the skin decreases in about 3.8 times in an hour after agent injection and then increases gradually, that shows the beginning of *glucose* diffusion from the observed area and corresponding reduction of tissue immersion. On the basis of the experiments it can be concluded that partial matching of refractive indices of collagen fibers of dermis (n=1.46) and interstitial medium (initially n=1.36) under action of 40%-*glucose* solution (n=1.396) makes the main contribution to tissue clearing.

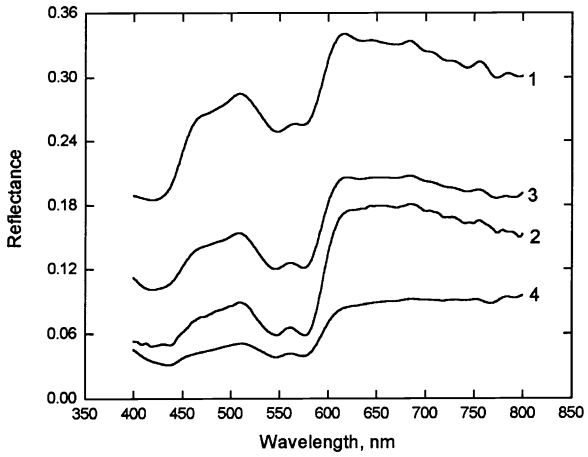

Fig. 2: The *in vivo* reflectance spectra of the human skin measured at administering of 40%-*glucose* solution in different time intervals: 1 – before injection, 2 – 3 min, 3 – 23, 4 - 60 min after hypodermic injection.

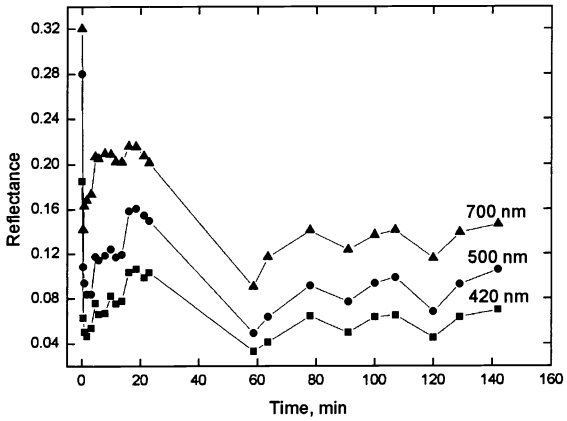

Fig. 3: The *in vivo* time-dependent reflectance of the human skin measured for different wavelengths at administering of 40%-*glucose* solution.

Obtained results have allowed estimating of diffusion coefficient of 40%-*glucose* solution in the human skin as $D_g = (2.56 \pm 0.13) \cdot 10^{-6}\ cm^2/\text{sec}$. The value of the diffusion coefficient has been calculated by the method described in Ref. 5. It is in about 2 times smaller then diffusion coefficient of *glucose* in water at 37^{0}C, $D \approx 5.2 \cdot 10^{-6}\ cm^2/\text{sec}$ and reflects the permeability of the collagen fiber network of the dermis [13].

It must be noted that skin was transparent during a few hours. From obtained value of *glucose* solution diffusion coefficient in the skin it can be estimated the time of impregnation of dermis layer with thickness 0.9 mm by *glucose*

solution, $\tau = l^2 / D_g \approx 53$ min. It is the time of *glucose* solution diffusion from the area of injection up to the epidermis, i.e. the time of skin clearing. It was observed experimentally.

The second phase is connected with taking down of the matching effect. It is determined by diffusion of *glucose* solution along the skin surface and in the depth.

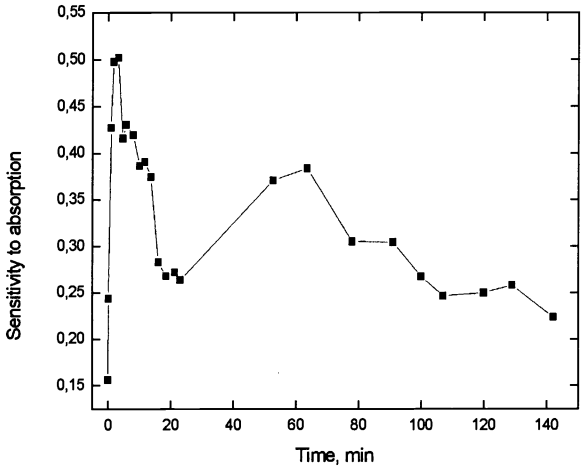

Fig. 4: Sensitivity to absorption of blood in dependence on time of clearing.

Dependence of the sensitivity to absorption of blood on time of clearing is shown in Fig. 4. The sensitivity to absorption of blood was calculated by $(R_{max} - R_{min})/(R_{max} + R_{min})$, where R_{max}, R_{min} are maximal and minimal values of skin reflectance in the wavelength region 400 – 800 nm, respectively. It is seen that contrast of blood in tissue increased in the first hour in correspondence with dynamics of reflectance change. The probe of skin was carried out on the depth of about 0.9 mm, which corresponds to the depth of *glucose* injection. So, due to the dermis immersion there is possible to increase substantially the imaging contrast of skin blood-perfused abnormalities.

3.2. *In vitro* study of the dye diffusion into the skin

In vitro experiments demonstrated the diffusion of dye dissolved in the osmotic liquids through rat skin tissue. Photographs of skin samples impregnated by 40%-*glucose* solution or *glycerol* for different periods of time are presented in Figs. 5 and 6, respectively.

The figures show that dye diffused from the site of the dermis. There were well seen the dyed areas of the samples. Dye penetration through the epidermis is limited due to the protective nature of the stratum corneum. Besides, different action of *glucose* solution and *glycerol* on the tissue was observed. *Glucose* solution causes the tissue swelling as it has subacid reaction (*pH* of 40%-*glucose* solution has been measured as 3.5). Thickness of the tissue increased. In 24 hours the tissue was completely dyed by *Methylene Blue* and the tissue swelling was observed (Fig. 5). *Glycerol* is neutral agent (*pH* = 6.5) and it causes high dehydration of tissue [3]. In Fig. 6 it is seen that thickness of the skin samples is decreased. The samples became rigid and *Methylene Blue* dye did not penetrate deeply into the sample during 24 hours.

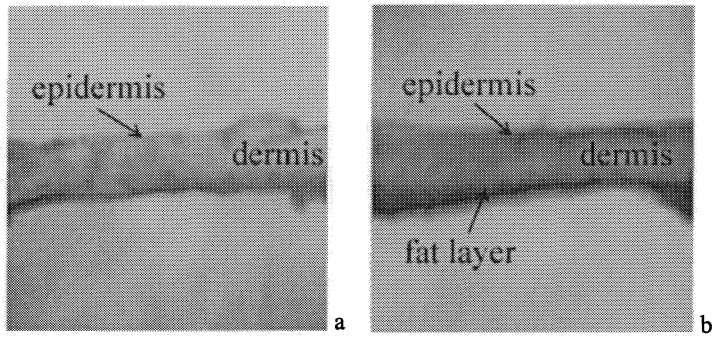

Fig. 5: Diffusion of *Methylene Blue* dissolved in 40%-*glucose* solution into the skin: a – image of skin slab after 205 minutes of acting; b – after 24 hours of acting.

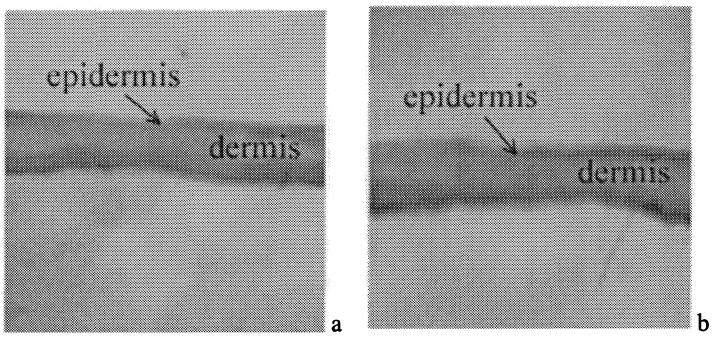

Fig. 6: Diffusion of *Methylene Blue* dissolved in *glycerol* into the skin: a – image of skin slab after 190 minutes of acting; b – after 24 hours of acting.

Thus *glucose* solution was choused as an active agent for the *in vivo* experiment. Administering of the agent through epidermis is not suitable for effective and fast clearing of skin therefore the injection of the active agent *in vivo* was done hypodermically to exclude the influence of the stratum corneum barrier.

4. CONCLUSION

The results of this paper show that administering of osmolytes to a fibrous tissue allows for effective control of the scattering properties of the skin, which are substantially reduced by the refractive indices matching of the scatterers and interstitial substance. For the first time the diffusion coefficient of 40%-*glucose* solution in the human skin has been estimated, $D_g = (2.56 \pm 0.13) \cdot 10^{-6}\ cm^2/\sec$. The most effective technique for an agent administering is hypodermic injection. Return back to the initial (turbid) state takes place very slowly (in a few hours) that allows one to carry out diagnostics and treatment of malignant growths hidden under the skin surface. The highest contrast of blood content abnormalities is expected after an hour after *glucose* injection.

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